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Detoxification of Organophosphate Pesticides Using a Nylon Based Immobilized Phosphotriesterase from *Pseudomonas diminuta*

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ABSTRACT

A partially purified phophostriesterase was successfully immobilized onto nylon 6 and 66 membranes, nylon 11 powder, and nylon tubing. Up to 9000 U of enzyme activity was immobilized onto 2000 cm^2 of a nylon 6 membrane where 1 U is the amount of enzyme necessary to catalyze the hydrolysis of 1.0 μmol of paraoxon/min at 25°C. The nylon 66 membrane-bound phosphotriesterase was characterized kinetically where the apparent K_m value for the immobilized enzyme was 0.35 m*M*. This is 5–6 times higher than that observed for the soluble enzyme. However, nylon immobilization limited the maximum rate of paraoxon hydrolysis to less than 10% of the value measured for the soluble enzyme. The addition of the cosolvent, methanol, resulted in an increase in the apparent K_m value for paraoxon hydrolysis but concentrations up to 40% had no negative effect on the catalytic effectiveness with the soluble or immobilized phosphotriesterase. Based on the kinetic analysis, methanol appears to be a competitive inhibitor for both forms of enzyme. The nylon powder immobilized enzyme was shown to be stable for at least 20 mo. The immobilization of the phosphotriesterase onto nylon provides a practical method for the detoxification of organophosphate pesticides.

Index Entries: Phosphotriesterase; organophosphate detoxification; immobilized enzyme.

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INTRODUCTION

A class of synthetic organophosphate compounds was discovered as useful pesticides and potential chemical warfare agents in the 1930s by Schrader (*1*). The use of organophosphate pesticides, though very important to the success of the agricultural industry, affects the environment. Notable public concern has been generally directed at the remaining pesticide residues in food products and the contamination of water supplies.

In addition to effects from direct pesticide usage, satisfactory methods for destroying pesticide residues in spray tanks and storage containers are limited. Current practices rely on landfills, chemical treatment, and incineration, with each having several disadvantages (*2,3*). A more convenient and reliable method for degrading these residues is required. The existence of structurally related chemical warfare agents is also a serious concern, both from the potential threat in warfare and the large stockpiles. Recent congressional legislation has demanded the reduction of these stockpiles, which in the United States is estimated at 25,000 tons (*4*). At present, incineration remains the most viable method for detoxification but potentially toxic emissions have raised environmental concerns and the construction of additional incinerators has met public opposition (*5*). Clearly, alternative methods for the detection and degradation of the organophosphates are needed.

The utilization of enzymatic reactions for the degradation of hazardous organophosphates has received some attention (*6,7*). Recently, an enzyme has been purified and characterized as a phosphotriesterase and catalyzes the hydrolysis of organophosphotriesters. This enzyme possesses a very broad substrate specificity, which includes a large number of organophosphate pesticides, such as parathion, diazinon, dursban, and coumaphos (*8*), in addition to chemical warfare agents, sarin and soman (*9*).

A feasible method for the degradation of these organophosphate compounds by the phosphotriesterase in a continuous fashion requires its immobilization onto a solid support. Recently, a partially purified phosphotriesterase was immobilized onto trityl agarose via physical absorption, and was proficient in hydrolyzing organophosphate pesticides (*6*). However, one major limitation of this immobilized enzyme system involved the use of organic solvents necessary to improve the solubility of the pesticides. Increasing methanol concentrations led to a desorption of enzyme activity from the bioreactor since this immobilization was based on hydrophobic interactions (*6*). To avoid such problems, covalent immobilization of this enzyme onto a solid support may serve as a reasonable alternative. There are numerous methods for the covalent attachment of proteins onto solid supports (*11*). Of the many solid support materials available, nylon offers several advantages. It is available in many different forms and it is physically stable to a wide variety of environmental

conditions. In this work, the phosphotriesterase was covalently immobilized onto several physical forms and types of nylon, and the capability of these immobilized enzyme systems to catalyze the hydrolysis of organophosphate pesticides is described.

MATERIALS AND METHODS

Materials

Phosphotriesterase was purified to a specific activity (SA) of 2000 U/mg of protein from *E. coli* cells carrying the cloned gene on a plasmid (*12*).† Nylon 11 powder (140 mesh) (Corvel Black) was obtained courtesy of Morton Thiokol, Inc., Specialty Chemicals Group, Reading, PA. The nylon 66 membrane was obtained from Schleicher and Schuell, Inc. The nylon 6 capsule (Nylafo filter) was purchased from Gelman Science, and the nylon 6 tubing was obtained from Cole Parmer, Inc. The glutaraldehyde, triethanolamine (TEA),2-[*N*-cyclohexylamino] ethanesulfonic acid (CHES), diisopropyl fluorophosphate (DFP), and paraoxon were purchased from Sigma Chemical Company. Ethyl parathion was purchased from Chem Service, Inc. and sodium borate was obtained from Fisher Scientific.

Preparation of the Nylon Tube Supported Phosphotriesterase

Immobilization of the phosphotriesterase was conducted according to a modified procedure of Inman and Hornby (*10*). A 3 m length of nylon tubing (1.6 mm id) was coiled around a plastic rod with a diamter of 1.2 cm and then was filled with an aqueous methanol (18.6% water/methanol) solution of $CaCl_2$ (18.6% by wt). The tubing was sealed at both ends and incubated at 50°C for 25 min. After rinsing the tubing with 250 mL of water, the tube was treated with 3.6*M* HCl at 45°C for 40 min at a flow rate of 5 mL/min and then washed with 250 mL of water at the same rate. A solution of 12.5% glutaraldehyde in 0.1*M* sodium borate buffer at pH 8.5 was then allowed to flow through the tubing at 2.5 mL/min at 0°C for 30 min. The tubing was washed with 50 mL of the same buffer for 5 min and then 1400 U of the phosphotriesterase were passed through the tubing at 2.5 mL/min at 0°C for 4 h in a closed loop. The tubing was then washed with 150 mL of 50 m*M* TEA at pH 9.0. The washes were analyzed for enzyme activity and 969 U of enzyme activity were recovered. Based on this analysis, 434 U of the enzyme were immobilized onto the nylon 6 tubing.

†This bacterial strain and plasmid are available from James Wild, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas.

Preparation of the Nylon Powder Supported Phosphotriesterase

Enzyme immobilization onto the nylon powder was accomplished according to a modified procedure of Inman and Hornby (*10*). A 6 mL solution of 12.5% glutaraldehyde in 0.1*M* sodium borate buffer at pH 8.5 was added to 750 mg of nylon 11 powder and stirred for 2 h at 4°C. The suspension was filtered through a glass sintered funnel and washed with 200 mL of the same buffer. The nylon 11 powder was then suspended in 15 mL of 0.1*M* sodium borate buffer and 240 U of the phosphotriesterase were added and the mixture slowly stirred at 4°C for 2 h. The suspension was poured into 50 mL of 100 m*M* CHES at pH 9.0. After filtering, no enzyme activity was detected in the effluent, indicating that 240 U of the phosphotriesterase were immobilized. A fixed bed reactor (0.7×6.5 cm) was constructed by pouring the nylon powder immobilized phosphotriesterase into a glass column. In a similar procedure, 900 U of the phosphotriesterase were immobilized onto 2.5 g of nylon 11 powder starting with 1200 U of enzyme activity.

Preparation of Nylon Membrane Supported Phosphotriesterase

Several nylon 66 membrane immobilized enzyme reactors were prepared according to a modified procedure of Inman and Hornby (*10*). The general procedure involved a nylon 66 circular membrane (4.5 or 7.6 cm in diameter, 0.45 μm thick) treated with 3 mL of a 12.5% glutaraldehyde solution in 0.1*M* sodium borate at pH 8.5, at 4°C for 2 h. The membrane was washed by immersing in a sodium borate solution and then treated with 3 mL of 400 U of the phosphotriesterase in the sodium borate buffer and allowed to stand for 2 h. The membrane immobilized enzyme was then washed by immersing in a solution of 0.1*M* sodium borate. The combined wash solutions and the remaining initial solution of phosphotriesterase were tested for enzyme activity. Based on this analysis, approx 180–200 U were immobilized (the range obtained from several repeated immobilizations). The nylon membrane immobilized enzyme bioreactor was constructed by fitting the nylon membrane into an 100 mL concentrator (4.5 cm diameter×7 cm).

Preparation of the "Nylaflo" Membrane (Nylon 6) Supported Phosphotriesterase

A 100 mL solution of 12.5% glutaraldehyde on 0.1*M* sodium borate at pH 8.5 was poured into a "Nylaflo" filter device and stored on ice at 4°C for 2 h while occasionally rotating the filter. The solution was then drained from the filter and approx 13,000 U of phosphotriesterase activity was

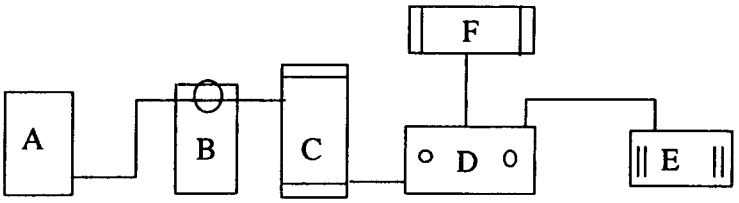

Fig. 1. A general schematic for the experimental system (**A**) substrate reservoir, (**B**) Rainin or Cole Parmer peristaltic pump, (**C**) reactor (fixed bed, continuous stirred batch, membrane, or tube), (**D**) Gilson Model HM Holochrome UV-Vis detector, (**E**) effluent collector and (**F**) chart recorder.

loaded into the "Nylaflo" filter. The enzyme solution was circulated through the filter for 16 h and then drained. The filter was washed with 0.1*M* sodium borate. A 20 m*M* CHES solution at pH 9.0 was then used to wash the filter at a continuous flow rate until less than 0.05 U/mL was detected. The enzyme activity in the collected washes indicated that approx 9000 U of the phosphotriesterase were immobilized.

Determination of Paraoxon Solubility in Methanol

One mL of a 83 m*M* solution of paraoxon in varied concentrations of methanol (0–40%) was vortexed vigourously and centrifuged. The concentration of the dissolved paraoxon was determined by removing and enzymatically hydrolyzing an aqueous paraoxon containing aliquot and then measuring the *p*-nitrophenol concentration spectrophotometrically at 400 nm ($\epsilon = 17{,}000/M$). This process was repeated several times and consistent results were obtained and are as follows: % methanol, m*M* paraoxon (0, 8.0; 10, 12.5; 20, 17.0; 30, 27.7; 40, 56.0), where increasing methanol concentrations led to a greater paraoxon solubility.

Activity Measurements

Enzyme activity is defined in units where 1 U is the amount of enzyme required to catalyze the hydrolysis of 1 μmol of paraoxon in 1 min. The experimental system used for the activity and kinetic determinations is illustrated in Fig. 1. The amount of enzyme activity was determined by monitoring the absorbance of the *p*-nitrophenol at 400 nm ($\epsilon = 17{,}000/M$), produced by the hydrolysis of paraoxon at pH 9.0 with a Gilford 260 spectrophotometer. Where applicable, the variation of methanol concentration on the extinction coefficient was considered in the hydrolysis rate determinations. The hydrolysis of the organophosphates in all cases was performed at constant flow rates and the fractions collected were used to determine the concentration of the hydrolysis product. The hydrolysis of

ethyl parathion was measured spectrophotometrically at 400 nm for the appearance of *p*-nitrophenol. The hydrolysis of DFP was determined using an Orion Model 94-09-00 flouride-sensitive electrode by measuring the concentration of the fluoride anion.

Kinetic Analysis

Kinetic measurements were made with the nylon 66 immobilized phosphotriesterase reactor at 25°C using paraoxon as the substrate. Steady-state hydrolysis rates were measured by monitoring the concentration of *p*-nitrophenol in the effluent at various flow rates with a Gilson Model HM Holochrome detector equipped with a 40 μL cuvet and a Linear chart recorder. Flow rates were kept constant with a Rainin peristaltic pump. Fractions were collected and the amount of *p*-nitrophenol produced at pH 9.0 was measured at 400 nm with a Gilford 260 spectrophotometer.

Data Analysis

Kinetic data were analyzed using the least squares method of Cleland (*13*). Data were fit to the appropriate equation to give the kinetic parameters and estimates of the standard errors. The apparent values for K_m and V_s were calculated from a fit to the data in the following equation

$$v = (V_s A) / (K_m + A)$$

where v is the initial velocity in μmol/min, V_s is the highest possible velocity in μmole/min, A is the initial paraoxon concentration, and K_m is the apparent Michaelis constant.

RESULTS AND DISCUSSION

Immobilization of Phosphotriesterase

The phosphotriesterase was successfully immobilized onto several forms of nylon, nylon 6 and 66 membranes, nylon 11 powder, and nylon 6 tubing. There are several chemical methods available for the immobilization of proteins onto nylon (*14,15*). The simplest and most direct method was chosen for the immobilization of the phosphotriesterase. This procedure involves a reaction of glutaraldehyde with the free amino groups of the nylon polymer followed by the coupling of the enzyme to the aldehyde functionality. However, the chemistry is believed to be more complicated (*16*). In the case of the nylon tube, acid hydrolysis was used to generate addditional free amino groups. Acid hydrolysis was not attempted with the nylon membrane owing to the physical nature of the thin membrane. The potential catalytic activity of the immobilized phosphotriesterase is defined as the amount of unrecovered enzyme activity following the

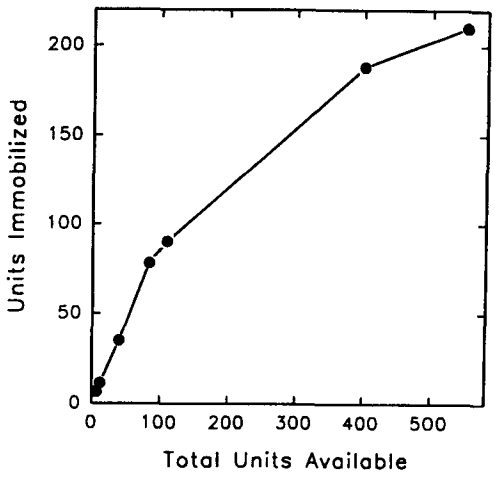

Fig. 2. Limits of bound phosphotriesterase on a nylon 66 membrane (4.5 cm circular membrane).

immobilization procedure relative to the initial activity of the soluble enzyme before immobilization. No enzyme activity was ever detected in the effluent during the utilization of the nylon-based immobilized phosphotriesterase. Figure 2 reflects the limiting amount of approx 200 U of phosphotriesterase activity that could be immobilized onto the nylon 66 membrane (4.5 cm in diameter). The proportion of the potential enzyme activity relative to the bound activity decreases as the amount of available soluble enzyme increases. However, utilization of a larger membrane, thereby increasing the available membrane surface area (and the free amino groups) from 28.3 to 47.7 cm^2 thus results in an increase in the amount of immobilized enzyme (390 U). Another limitation on the amount of immobilized enzyme may also result from the utilization of a partially purified phosphotriesterase, and therefore, available sites may be occupied by contaminating proteins. Improvements in the amount of enzyme activity that can be bound may be possible by utilizing other chemical methods (*14*).

Nylon 66 Membrane

The covalent attachment of the phosphotriesterase to the nylon 66 membrane was a simple and convenient operation. The effect of flow rate and substrate concentration on the capability of the immobilized phosphotriesterase to hydrolyze paraoxon is depicted in Fig. 3. With the 11-U reactor, faster flow rates at any given substrate concentration resulted in lower concentrations of hydrolyzed product. However, with increasing substrate concentration, the quantity of paraoxon hydrolyzed per minute increases at a given flow rate. A noticeable improvement in the effective hydrolysis of paraoxon is observed by increasing the concentration of

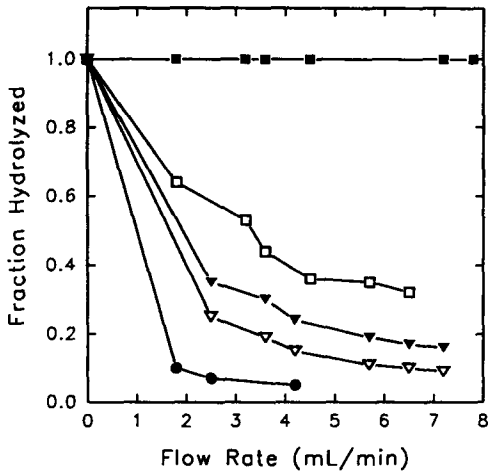

Fig. 3. The hydrolysis of paraoxon in 50 m*M* CHES at various flow rates with the nylon 66 membrane immobilized phosphotriesterase (11 U) (□, 0.12 m*M*; ▼, 0.5 m*M*: ▽, 0.1 m*M*; ●, 4.0 m*M*); (180 U) (■, 4.0 m*M*).

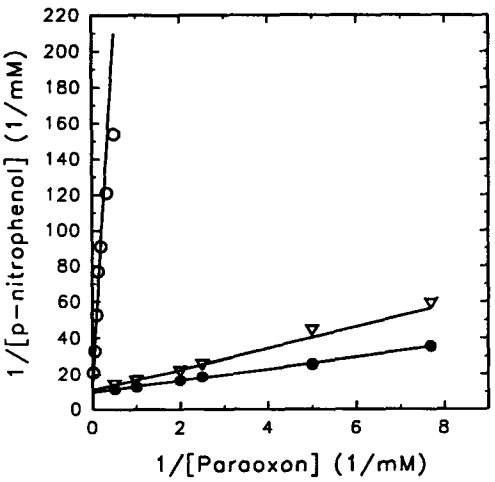

Fig. 4. Double reciprocal plots for the nylon 66 membrane immobilized phosphotriesterase (11 U) with varying concentrations of paraoxon at 0% (●), 10% (▽), and 40% (○) methanol concentrations.

immobilized enzyme to 180 U. A near quantitative hydrolysis of a 4.0 m*M* paraoxon solution demonstrates that higher substrate concentrations can be hydrolyzed as faster flow rates by increasing the immobilized enzyme concentration.

The 11-U membrane reactor has also been characterized kinetically with and without methanol as a cosolvent. The data are plotted as double reciprocal plots, which are presented in Fig. 4. The linearity of these plots

Table 1
The Kinetic Parameters for the Hydrolysis of Paraoxon[a]

% Methanol	K_m mM	V_s μmol/min
Immobilized phosphotriesterase		
0	0.35±0.02	0.46±0.01
10	0.55±0.04	0.42±0.01
40	30.2±3.3	0.35±0.01
Soluble phosphotriesterase		
0	0.06±0.01	11±1[b]
10	0.20±0.01	12±1
40	4.5±0.5	11±1

[a]With an 11-unit immobilized nylon 66 membrane reactor and the soluble phosphotriesterase at varied concentrations of methanol.
[b]Using 11 units of soluble enzyme.

demonstrates that Michaelis Menten-type kinetics are observed in this concentration range and the corresponding kinetic parameters derived from these plots are presented in Table 1. For the 11-U membrane reactor in the absence of methanol, the apparent K_m value is 0.35 m*M*, and the catalytic efficiency is reduced to about 4% of the soluble enzyme (0.46/11). An increase in the flow rate to 7.2 mL/min resulted in a decrease in the K_m value to 0.27 m*M* with no substantial change in V_s (not shown). This reduction is minimal, but it does suggest a decrease in external diffusion limits resulting from an increase in the flow rate. The apparent K_m values for the immobilized phosphotriesterase, although 5–6 times higher, are comparable in magnitude to that for the soluble enzyme (K_m = 0.06 m*M*). Larger K_m values are not unusual for immobilized enzyme systems and are rationalized on the diffusional limits of the substrate's access to the enzyme active site (*17,18*). Other arguments attribute increases in K_m values to steric and conformational constraints (*17,18*). Unlike the trityl agarose immobilized phosphotriesterase, there should be no substantial substrate affinity with the nylon membrane, since the substrates are neutral and the nylon surface is relatively hydrophilic.

The limited efficiency of 4% for this nylon membrane immobilized phosphotriesterase may be explained, in part, by the very high catalytic activity of the soluble phosphotriesterase with paraoxon where the encounter of paraoxon at the active site is rate limiting (*19*). Internal diffusional effects also have been observed to reduce the catalytic efficiency of immobilized enzymes (*17,18*) and may also be operating in this bioreactor. Finally, it is unknown to what extent the immobilization process affects the initial soluble enzyme activity. Glutaraldehyde has been shown to stabilize enzymes by crosslinking at low concentrations, but at the high concentrations (>3%), excessive crosslinking can result in denaturation of the enzyme (*20*).

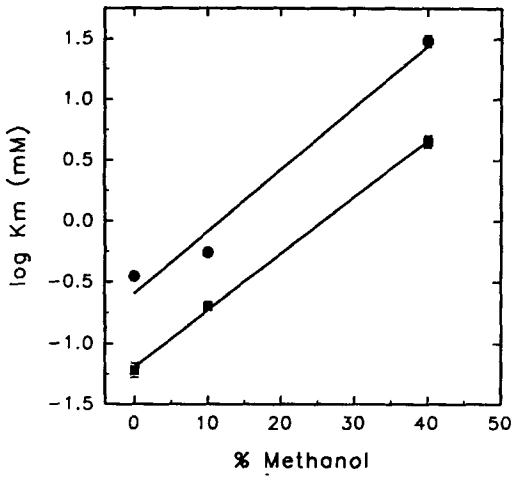

Fig. 5. The effect of methanol on the kinetic parameter, K_m, for the hydrolysis of paraoxon with the soluble (●) and the nylon 66 membrane immobilized phosphotriesterase (11 U) (■).

The degradation of higher concentrations of pesticides is advantageous and would ultimately reduce volumes of total effluent. Achieving higher concentrations of pesticides is possible with the incorporation of a cosolvent, such as methanol. Unfortunately, many proteins are subject to denaturation or precipitation in organic solvents, especially at high concentrations. The use of methanol with an immobilized parathion hydrolase was found to have adverse effects on the catalytic activity (7). The effect of methanol on enzyme activity was studied utilizing the soluble form of the phosphotriesterase. The phosphotriesterase activity did not decrease with increasing concentrations of methanol up to 40%, and at 60% methanol concentrations, a 30–40% decrease in enzyme activity was observed over a 15 h time-span. These results indicate that for the soluble enzyme, there are no deleterious effects of methanol up to concentrations of 40%, thus allowing solubilities of paraoxon to approach 56 m*M*. Concentrations greater than 80% methanol resulted in protein precipitation and enzyme activity was reduced by 80–90%.

The effect of methanol as a cosolvent at varied concentrations was examined using the nylon 66 membrane reactor and the kinetic parameters were determined and compared to those of the soluble enzyme. The kinetic parameters at 10% and 40% methanol concentrations are presented in the reciprocal plots in Fig. 4 and given in Table 1. Increasing K_m values are observed with increasing methanol concentrations for both the immobilized and soluble form of the enzyme with the K_m values for the immobilized form higher at each methanol concentration. The effect of increasing methanol concentration on the apparent K_m is graphically depicted in Fig. 5. Although the data are limited, the parallel slopes between the immobil-

ized and soluble form of the enzyme suggest that the effect of methanol on both forms of the enzyme is similar. The common V_s and relative V_{max} values at various concentrations of methanol for the bound and soluble form of the enzyme, respectively, suggest that methanol is a competitive inhibitor. Previous studies with the soluble phosphotriesterase indicated that methanol was a competitive inhibitor ($K_i = 0.64M$) (*21*). These results affirm that concentrations of methanol up to 40% can be utilized for the hydrolysis of insoluble organophosphate pesticides with no effect on the catalytic efficiency of the nylon immobilized phosphotriesterase. Furthermore, higher concentrations of methanol allow the detoxification of reduced volumes of high substrate concentrations, and thereby limit the amount of effluent that must be handled. Methanol was chosen as a cosolvent because DMF and DMSO are much more inhibitory (*21*). Unlike the trityl agarose immobilized phosphotriesterase, no enzyme activity was detected in the effluent with the nylon 66 immobilized enzyme and steady-state hydrolysis rates of paraoxon were possible for at least 1 h at 25°C with concentrations of at least 40% methanol. Repeated use of this 11-U membrane reactor over a 4-wk period did not appear to affect the catalytic efficiency for the hydrolysis of paraoxon.

The 390-U nylon 66 membrane (7.6 cm in diameter) was utilized to test the ability of the enzyme to hydrolyze the same substrates as the soluble enzyme. A 4.0 m*M* paraoxon solution and a 0.2 m*M* solution of ethyl parathion were quantitatively hydrolyzed, whereas a 2.5 m*M* solution of DFP, a poorer substrate, is hydrolyzed to only 34%. Therefore, the nylon membrane immobilized phosphotriesterase is capable of hydrolyzing organophosphate pesticides and chemical warfare-like agents.

"Nylaflo" Membrane Filter

Based on the observation that the immobilization of the phosphotriesterase is limited, in part, by the available surface area and that increasing the bound activity leads to increased hydrolysis rates, a "Nylaflo" filtering device (nylon 6 membrane) containing 2000 cm^2 of surface area was employed as a potential enzyme reactor. Approximately 9000 U of the phosphotriesterase activity were successfully immobilized onto the nylon 6 membrane. Unlike the immobilizations of the other membranes, the glutaraldehyde solution could not be totally rinsed from the inner core of the filtering device and, therefore, crosslinking as well as covalent attachment of the phosphotriesterase are highly probable. In addition, the use of this filtering device allowed very high flow rates, up to 155 mL/min with connecting tubing having an id of 0.8 cm. Total hydrolysis of a 5.5 m*M* paraoxon solution was achieved at 155 mL/min with a hydrolysis rate of about 850 μmole/min. Because the total hydrolysis of this paraoxon solution was possible and knowledge of the kinetic parameters does

not exist, it is difficult to conclude that this represents the maximum effective hydrolysis rate. This value is however, consistent with the 4% efficiency observed with the smaller nylon 6 membrane. Although this system was not fully characterized, it demonstrates the capability to increase enzyme immobilization by increasing the available surface, which results in the improved catalytic potential and shows that dilute solutions of organophosphate pesticides can be effectively degraded.

Nylon Powder Immobilized Phosphotriesterase

A total of 240 U of phosphotriesterase activity was successfully immobilized onto 750 mg of nylon 11 powder (140 mesh). The 240-U nylon immobilized phosphotriesterase was used to construct a fixed bed reactor, which showed the ability to hydrolyze 1.0 mL of a 18.3 m*M* paraoxon solution in 30% methanol, where complete hydrolysis was accomplished at 0.57 mL/min with total recovery of the hydrolyzed product. Flow rates with this fixed bed reactor were limited to 0.7 mL/min because of back pressure resulting from the fine mesh of the nylon powder. However, although limiting the flow rate, the small mesh size of the nylon powder offers the advantage of providing more available surface area for enzyme immobilization. The storage stability of this immobilized enzyme was also examined and after 20 mo of standing at 5°C, the immobilized phosphotriesterase was again tested for its ability to hydrolyze paraoxon. At a flow rate of 0.7 mL/min, a 18.7 m*M* paraoxon solution in 30% methanol was hydrolyzed completely with no apparent affinity of the substrate for the nylon support. Therefore, this form of nylon immobilized phosphotriesterase appears stable for long periods of time and is capable of hydrolyzing significant concentrations of paraoxon in high amounts of methanol.

Other potential applications of the nylon powder were considered when 900 U of enzyme activity were immobilized onto 2.5 g of the nylon powder. This reactor demonstrated the ability to hydrolyze a 18.3 m*M* paraoxon solution in 30% methanol at 76 μmol/min. However, because the kinetic parameters were not determined for this reactor, this measured hydrolysis rate may not represent the maximum potential of this system. Although product inhibition may become important for a high degree of substrate hydrolysis with this type of reactor (22) and total enzyme recovery may technically be reduced in the filtration process, this type of reactor is very simple to operate and maintain, and may allow for more practical applications.

Nylon Tubing

Immobilization onto nylon tubing has been used as a quantitative detection device with other enzymes (23). The immobilization of the phosphotriesterase onto nylon 6 tubing involves the pretreatment of the

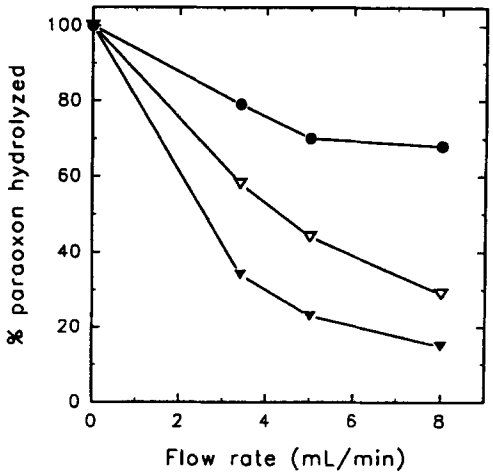

Fig. 6. The hydrolysis of paraoxon in 100 m*M* CHES at various flow rates with nylon tube immobilized phosphotriesterase (430 U) (●, 0.1 m*M*; ∇, 1.0 m*M*; ▼, 2.0 m*M*).

inner tubing surface with a calcium chloride/methanol solution, thereby increasing the surface area for enzyme immobilization (*10*). Of the 1400 U of the phosphotriesterase available for immobilization, only 430 U were not recovered and presumed immobilized. There was no detectable activity in the effluents following the initial wash. This immobilized enzyme form was capable of hydrolyzing paraoxon as shown in Fig. 6, where increasing flow rates resulted in a decrease in the hydrolysis rate at each concentration of paraoxon. An initial kinetic analysis of this data revealed a nonlinear reciprocal plot (not shown) typical of previously reported nylon tube immobilized enzymes (*24*). Because much higher flow rates and higher concentrations of paraoxon were required to determine the kinetic parameters, a complete kinetic analysis was not performed. However, the immobilization onto the nylon tube does result in another functional method for the degradation of organophosphate pesticides.

CONCLUSIONS

The immobilization of the phosphotriesterase onto several physical forms of nylon provides an effective method for the catalytic degradation of organophosphate pesticides. The kinetic parameters for the nylon membrane immobilized enzyme are comparable to those of the soluble enzyme although the catalytic effectiveness is significantly reduced for the membrane-bound phosphotriesterase. The addition of a cosolvent to concentrations of at least 40% methanol did not affect the catalytic ability

of this immobilized enzyme and, therefore, allows very high substrate concentrations to be degraded. Very high enzyme concentrations can be bound by increasing the overall available surface area and thus higher effective hydrolysis rates can be achieved. The versatility offered by the various physical forms of the nylon solid support now provide feasible methods for the detection and degradation of organophosphate pesticides.

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